

**Remarks**

Applicants have amended the specification to insert reference to the sequence listing and to correct minor typographical error. In accordance with the provisions of 37 C.F.R. §1.121(b)(1)(iii), attached herewith as Appendix A is a marked-up copy of the specification indicating the amendments.

Applicants note that none of the above amendments are being made for any reasons related to patentability

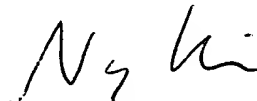
None of the above amendments adds any new matter to the Application as filed.

**Conclusions**

No additional fees are believed to be due in connection with this filing. However, please charge any underpayments or credit any overpayments to our Deposit Account No. 08-0219.

If there are any questions, please call the undersigned at the telephone number indicated below.

Respectfully submitted,  
HALE AND DORR LLP



Nancy Chiu, Ph.D.  
Reg. No. 43,545  
Agent for Applicant

Date: November 2, 2001

60 State Street  
Boston, MA 02109  
(617) 526-6000  
(617) 526-5000 (fax)

## APPENDIX A

### Marked-up Version of the Amended Specification Pursuant to 37 C.F.R. §1.121(b)(2)(iii)

At page 11, lines 4-7:

Figure 2 shows a direct amino acid sequence comparison of the mannose lectin described by Gowda et al. (*J. Biol Chem* 269:18789-18793, 1994: SEQ ID NO:49 and SEQ ID NO:51) and the derived amino acid sequence of DI-FRIL, a representative, non-limiting FRIL family member of the invention (SEQ ID NO:50 and SEQ ID NO:52), encoded by a representative, non-limiting nucleic acid of the invention.

At page 11, lines 23-25:

Figure 9 is a map of a recombinant expression vector pGEX4T-1-DLA manufactured by ligating a wild-type cDNA clone in the *EcoRI/SalI* site of the *E. coli* expression vector pGEX4T-1 (insertion site as SEQ ID NO:53).

At page 11, lines 26-28:

Figure 10 is a map of a recombinant expression vector pGEX4T-1-DLA(D) manufactured by ligating a mutant cDNA clone in the *EcoRI/XhoI* site of the *E. coli* expression vector pGEX4T-1 (insertion site as SEQ ID NO:54).

At page 14, lines 23-26:

Figure 24B shows a direct amino acid sequence comparison of Pv-FRIL(SEQ ID NO:56), a representative, non-limiting FRIL family member of the invention, with DI-FRIL(SEQ ID NO:55), another representative, non-limiting FRIL family member of the invention, and the PHA lectin, PHA-E (SEQ ID NO:57).

At page 53, lines 8-12:

Based on the amino acid sequence published by Gowda et al., *J. Biol. Chem.* 269:18789-18793, 1994, two degenerate oligonucleotide primers were designed using *Phaseolus* codon usage (Devereux et al., *Nucleic Acids Res.* 12:387-394, 1984):

MLA AA(AG)TT(TC)GA(TC)CC(AT)AA(TC)CA(AG)GA(AG)GA (SEQ ID NO:[\_] 11)

MLZ TT(AT)CC(AG)TT(TC)TGCCA(AG)TCCCA (SEQ ID NO: [ ] 12).

At page 53, lines 19-23:

The 500 bp product obtained by PCR was cloned in the cloning vector, pCR2.1 (Invitrogen, Carlsbad, CA), and sequenced by sequenase dideoxy chain termination (United States Biochemicals) using the following primers:

GTACCGAGCTCGGAT (SEQ ID NO:[\_] 13)

TCTAGATGCATGCTCGAG (SEQ ID NO:[\_] 14).

At page 53, lines 26-28:

Based on the sequence of the D1-FRILa amplified product, a specific primer was prepared:

MLX GTTGGACGTCAATTCCGATGTG (SEQ ID NO: [ ] 15).

At page 54, lines 1-3:

A degenerate primer corresponding to the first five amino acids of the sequence published by Gowda et al., *J. Biol. Chem.* 269:18789-18793, 1994 was also prepared:

MLI GC(TC)CA(AG)TC(TC)CT(TC)TC(TC)TT (SEQ ID NO: [ ] 16).

At page 54, lines 16-19:

AP GACCACGCGTATCGATGTCGAC (SEQ ID NO: [ ] 17).

Nested PCR amplifications were performed using the AP anchor primer in combination with a specific primer having the following sequence:

MLB AAGTTAGACAGTGCAGGAAAC (SEQ ID NO: [ ] 18).

At page 54, lines 24-27:

To obtain the full length cDNA clone, the anchor primer AP was used in combination with a specific primer corresponding to the first 5 amino acids encoded at the 5'-terminus:

MLII            GCACAGTCATTGTCATTAG    (SEQ ID NO: [ ] 19).

At page 57, lines 6-13:

A comparative illustration of the derived DI-FRIL amino acid sequence with the reported amino acid sequence of the mannose lectin as determined by Gowda et al. (*J. Biol. Chem.* 269:18789-18793, 1994) is shown in Fig. 2. The single sequence derived for DI-FRIL protein comprises domains that correspond directly and with substantial homology to the  $\alpha$  subunit (SEQ ID NO: [ ] 52) and  $\beta$  subunit (SEQ ID NO: [ ] 50) of the protein described by Gowda et al., *supra*. When the  $\beta$  subunit of the Gowda et al. (*supra*) protein is assigned to the N-terminal domain and is followed linearly by the  $\alpha$  subunit, the arrangement of the polypeptides shows homology to other legume lectins.

At page 57, lines 21-25:

To establish functionality of homologs of the protein encoded by the DI-FRIL cDNA, a mutation was made in the DI-FRIL cDNA clone. The domains of the derived protein and the pea lectin that include the mutation site are shown below:

|         |   |                      |
|---------|---|----------------------|
| DI-FRIL | . Y L N P D Y G . D P N Y I H I G I D V | (SEQ ID NO: [ ] 20)  |
| Pea     | F Y . N A A W D P S N R D R H I G I D V | (SEQ ID NO: [ ] 21). |

At page 58, lines 1-7:

To introduce the mutation, recombinant PCR was performed (see, *e.g.*, Higuchi, R., *PCR Protocols: A Guide to Methods and Applications*, Innis M.A., Gelfand D.H., Sninsky J.J., and White T.J., eds., Academic Press, San Diego, 1990). Two PCR reactions were carried out separately on the full length cDNA using two primers that contain the same mutation and produce two products with an overlapping region:

|       |                          |                      |
|-------|--------------------------|----------------------|
| MutI  | CCATAATCGGGATCAAGATAGGTG | (SEQ ID NO: [ ] 25)  |
| MutII | CACCTATCTTGATCCCGATTATGG | (SEQ ID NO: [ ] 26). |

At page 58, lines 8-13:

The primary PCR products were purified with the QIAquick PCR Purification kit (QIAGEN, Valencia, CA), according to the manufacturer's instructions. The overlapping primary products were then combined and amplified together in a single second reaction using flanking primers:

|         |                              |                      |
|---------|------------------------------|----------------------|
| M1 Forw | AACTCAGCCGCACAGTCATTGTCA     | (SEQ ID NO:[ ] 27)   |
| APEcoRI | GAATTCGACCACGCGTATCGATGTCGAC | (SEQ ID NO: [ ] 28). |

At page 59, lines 7-10:

The following primers were used for amplification of the signal peptide sequence:

|         |                              |                     |
|---------|------------------------------|---------------------|
| Sigforw | GAATTCATGGCTTCCTCCAAC        | (SEQ ID NO: [ ] 29) |
| Sigrev  | TGACTGTGCGGCTGAGTTTGCGTGGGTG | (SEQ ID NO:[ ] 30). |

At page 59, lines 11-13:

The primers M1Forw (SEQ ID NO: [ ] 27) and APEcoRI (SEQ ID NO: [ ] 28) used for amplification of the DI-FRIL cDNA described above, were again used to amplify the DI-FRIL cDNA.

At page 74, lines 23-29:

Throughout purification of Pv-FRIL a New Zealand White rabbit (HRP, Denver, PA) was immunized with crude PHA-LCM, boosted with increasingly purified samples containing Flt3 3T3 activity, and finally immunized with a peptide corresponding to Pv-FRIL (AQSLSF[N, C, S]FTKFDLD; SEQ ID NO:31), referred to as the AQS-peptide. Samples were glutaraldehyde conjugated to keyhole limpet hemocyanin (KLH, Sigma). The rabbit was immunized with KLH-AQS peptide-containing samples using either Complete Freund's Adjuvant (Sigma) or Hunter's Titermax (Vaxcel, Inc.,

At page 78, lines 3-9:

In each of three experiments, the sequence AQSLSFXTKDALD (SEQ ID NO:32) was obtained from a polypeptide of 18 kDa (where X is an unknown amino acid). For the material at the dye front (14 kDa and below) the aminoterminal sequence of TDSRVVAVEFDXFP (SEQ ID NO:33) was found twice. The amino terminus of a smooth muscle protein (SM22- $\alpha$ ) was found twice and the amino terminus of myoglobin identified once. Since the sequence starting with AQS was the only sequence identified in each experiment, this polypeptide was concluded to be responsible for Flt3 3T3 activity.

At page 79, lines 5-12:

Because PHA is derived from red kidney bean extract and because a FRIL family member, DI-FRIL, was isolated from another legume, namely *Dolichos lab lab*, mannose-binding lectins were isolated from red kidney bean (*Phaseolus vulgaris*) extract using standard methods, such as the procedure of Rudiger, H., *Isolation of Plant Lectins*, H.-J. Gabius and S. Gabius, eds., pp. 31-46, Berlin, 1993). The kidney bean mannose-binding lectin consisted of polypeptides with molecular weights of 18 kDa and 15 kDa, and the aminotermini of these two polypeptides started with AQSLSFXTKFDPN (SEQ ID NO:34) [AND] and TDSRVVAVEDF (SEQ ID NO:35), respectively (where X is an unknown amino acid).

At page 80, lines 15-18:

**PVBeta1:** TTY ACY AAR TTY GAY YTN GA (SEQ ID NO:36)  
**PVBeta2:** ATY TTY CAR GGW GAY GC (SEQ ID NO:37)  
**PVAlfa1:** TTR ACR TCR ATW CCR ATR TG (SEQ ID NO:38)  
**PVAlfa2:** TAR TTW GGR TCR ATR TTR GCR TT (SEQ ID NO:39).

At page 81, lines 21-22:

**PV3:** CAA TGT CTT ACA ACT CAC TAA G (SEQ ID NO:40)  
**PV4:** AGT GTG GGA AGA GTG TTA TTC (SEQ ID NO:41).

At page 82, lines 4-5:

**SPV2:** ACC AAA GCT TTG GTT TTC AGA (SEQ ID NO:42)  
**SPV3:** TCT GAA AAC GTT TGA GTA GAG (SEQ ID NO:43).

At page 82, lines 21-22:

**PVEcoRI** TAC ATG AAT TCG CTC AGT CAT TAT CTT TTA AC (SEQ ID NO:44)  
**APExcoRI:** GAA TTC GAC CAC GCG TAT CGA TGT CGAC (SEQ ID NO:28).

At page 84, lines 13-19:

The primers used for the two primary reactions are the following:

Amplification of the Signal Peptide

**Sigforw BglIII:** AGA TCT ATG GCT TCC TCC AAC (SEQ ID NO: 45)  
**Sigrew:** AAA GAT AAT GAC TGA GCG GCT GAG TTT GCG TG (SEQ ID NO: 46).

Amplification of the mannose lectin cDNA:

**SpMlforw:** CAC GCA AAC TCA GCC GCT CAG TCA TTA TCT TT (SEQ ID NO: 47)  
**APXhoI:** CTC GAG GAC CAC GCG TAT CGA TGT CGA (SEQ ID NO:48).